

Comparative Study between Radiofrequency Ablation Combined with either Percutaneous Ethanol Injection or Percutaneous Acetic Acid Injection in the Management of Hepatocellular Carcinoma.

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Background and study aim: Radiofrequency ablation (RFA) has attracted the greatest interest because of its effectiveness and safety. However, complete tumor necrosis rate with RFA for tumors larger than 5 cm is less favorable. Acetic acid, which has the ability to penetrate cells with the property of extracting collagen and dissolving lipids, has a stronger killing effect on hepatocytes than ethanol. The aim of this study was to compare two percutaneous combined therapies, RFA plus percutaneous ethanol injection (PEI) and RFA plus percutaneous acetic acid injection (PAI) in hepatocellular carcinoma (HCC) treatment in patients having single focal lesions more than five and up to seven cm in diameter.

Patients and Methods: The patients were selected according to the triphasic CT scanning characteristics of HCC. They were randomly allocated into 2 groups: GI

included 30 patients who were candidates for 1 setting of RFA then PEI in the same setting. GII included 30 patients ;they were ablated by 1 setting of RFA with 2 overlaps to decrease the size of the remaining unablated areas, then PAI in the same setting.

Results: Evaluation of the response one month later using triphasic CT showed significant better responses in GII compared to GI (83.3% vs 60%) with P value <0.05. The response 3 months later was not changed significantly compared to the response after one month of the procedure (56.7% in GI and 80% in GII)

Conclusion: Ablation of inoperable large HCC 5.1-7cm can be done with combined techniques. RFA plus acetic acid was compared to RFA plus ethyl alcohol. Complete ablation was superior when acetic acid was used.

INTRODUCTION

HCC is the fifth most common neoplasm globally and the second most common cause of cancer-related death by the World Health Organization. The predomination of liver cancer is greater than 2:1 in male-to-female gender. In 2012, almost 83% of the estimated 782,000 HCC cases showed in less developed regions. East, South Asia and sub-Saharan Africa are the regions of highest incidence, whereas in Southern Europe and North America are the regions of the intermediate incidence, while Northern Europe and South-Central Asia being the regions

of lowest incidence [1]. HCV and HBV are the most common precipitating factors in HCC development. HCV has affected around 12% of the Egyptian population leading to cirrhosis in about 20%. In such patient HCC develops in about 15% of the cases [5]. In order to decide the appropriate therapeutic choice, the accurate diagnosis of neoplastic lesions by one or more of the imaging modalities is mandatory. This imaging workup has two purposes; lesion characterization and cancer staging [2]. Often, once HCC has been

detected, it is difficult to treat due to chemo-resistance, multicentric incidence and underlying poor hepatic reserve, which in itself leads to a high mortality rate [3]. Surgery offers the only real chance of cure, but the majority has an unresectable disease because of tumor stage or liver cirrhosis [4]. Local ablation is a safe and effective therapy for patients who cannot undergo resection or as a palliative treatment before transplantation [5]. Among the various local percutaneous ablative therapies, radio-frequency ablation (RFA) has attracted the greatest interest because of its effectiveness and safety in the treatment of small HCCs, with 62% - 68%, a 3-year survival rate, and related morbidity and mortality rates of 0%-12% and 0%-1% respectively. Although, less favorable results of complete tumor necrosis rate with RFA occurs in tumors larger than 5 cm in diameter, while the local recurrence rate can be as high as 20%, even for HCCs less than 3.5 cm in diameter [6]. Thus, to improve RFA results, the ablation zone needs to be extended, so that larger tumors can be treated and local recurrences of smaller tumors after treatment can be decreased [7]. Acetic acid, which has the ability to penetrate cells with the property of extracting collagen and dissolving lipids, has a stronger killing effect on hepatocytes than ethanol [8]. PEI is also effective in ablating small tumors [9].

The aim of the present study was to evaluate and compare two percutaneous combined therapies, RFA plus PEI and RFA plus PAI in HCC treatment in patients having single focal lesions more than 5 and up to 7 cm in diameter.

PATIENTS AND METHODS

This prospective study was conducted on 60 patients with compensated HCC who had single lesion 5 cm and up to 7 cm in diameter, they were Child A according to Child Pugh classification. Their ages ranged from 46 to 77. They were managed at RFA unit of Tropical Medicine Department, Al-Azhar University Hospitals from April 2014 to January 2017. Ethical approval was taken for conducting this study and written consents were obtained. The patients were selected according to the triphasic CT scanning characteristics of HCC (early uptake of the dye in the arterial and rapid washout in venous and delayed phases). They were randomly allocated into 2 groups: GI included 30 patients who were candidates for 1 setting of RFA then PEI in the same setting. Their mean age was 60.7 (Table 1).

GII included 30 patients. They were ablated by 1 setting of RFA with 2 overlaps to decrease the size of the remaining unablated areas, then PAI in the same setting. Their mean age was 58.1 (Table 1). According to the lesion size, each group was subdivided into 2 subgroups: GA which included 15 patients who had lesions 5.1 to 6 cm in maximum diameter and GB which included 15 patients who had lesions 6.1 to 7 cm in maximum diameter. All patients were subjected to pre-procedure work up including full history, thorough clinical examination, and detailed investigations. This was followed by performing triphasic CT scanning and Alpha-fetoprotein (AFP) 1 month and 3 months later. The results were compared to decide if one regimen was better than the other.

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. The following tests were done: Independent-samples t-test of significance was used when comparing the two means, Chi-square (X²) test of significance was used in order to compare proportions between two qualitative parameters and Probability (P-value). P-value <0.05 was considered significant, P-value <0.001 was considered as highly significant and P-value >0.05 was considered insignificant.

RESULTS

This study was conducted on 60 patients having single focal HCC, ranging from 5.1 to 7 cm in diameter. The mean age of the patients was 60.30. It was 60.70 for GI and 58.16 for GII. They 51 (85%) males and 9 (15%) females. GI composed of 26 males (86.7%) and 4 females (13.3%). GII composed of 25 males (83.3%) and 5 females (16.7%). The studied patients included 13 smokers (22%) as 6 (20%) in GI and 7 (23.3%) in GII (Table 1). No significant statistical difference was detected between the two groups. HCV Ab was positive in 54 (90%) patients, 26 (8.7%) for GI and 28 (93.3%). HBs Ag was positive in one patient in GI. Combined infection was seen in 3 (10%) patients of GI and 2 (6.7%) patients of GII (Table 2). No significant statistical difference was detected between the two groups. AFP was positive in 44 (73.3%) patients, 23 (7.6%) in GI and 21 (70%) in GII (Table 3). The difference was not significant between the

groups. When AFP levels were compared after one month of ablation a significant drop was observed. However, no further drop was noted 3 months later (Table 4). Evaluation of the response one month later using triphasic CT showed significant better responses in GII compared to GI (83.3% vs 60%) with P value <0.05. The response 3 months later was not changed significantly compared to the response after one month of the procedure (56.7% in GI and 80% in GII) (Table 5,6). When the groups were subdivided into two subgroups A and B. Subgroups GI A showed better results when compared to GI B (66.7% vs 53.3%). However, this did not reach a significant value after 1 month of ablation (P >0.05) (Table 9). This was also observed 3

months later (Table 10). Comparing subgroups GII A to GII B better results were in favor to GII A (Table 11 & 12). However, it did not reach a significant value (P <0.05). Comparing subgroup GI A to GII A (66.7% vs 86.6%) significantly better response was noted in GII. Comparing subgroup GI B to GII B (46.6 vs 73.3%) significantly better response was observed in GII B. The Complications reported were abdominal pain and fever. This was noted in 63.3% of patients, 60% in GI and 66.7% in GII. The difference was not significant. Fever was observed in 26.6% of patients, 23.3% for GI and 30% for GII. The difference was also not significant (Table 13). The results indicate significantly better responses when acetic acid was combined to RFA.

Table (1): Demographic features of the studied groups

	Group I	Group II	Total	t/x ²	P
Age (year)					
Mean	60.70	58.16	60.30	1.223	>0.05
SD	8.55	7.51	8.58		
Range	46- 77	48- 73	46-77		
Sex				0.084	>0.05
Male	26(86.7%)	25(83.3%)	51(85%)		
Female	4(13.3%)	5(16.7%)	9(15%)		
M: F ratio	7:1	5:1	6:1		
Smoking				0.004	>0.05
Smoker	6(20 %)	7(23.3%)	13(22%)		
Non- smoker	24(80 %)	23(76.7%)	47(78%)		

There is no significant difference of the mean values between the studied groups.

Table (2): Prevalence of HCV-Ab and HBsAg among the studied groups

	Group I	Group II	Total	X ²	P
HCV Ab	26 (86.7%)	28(93.3%)	54(90%)	1.884	>0.05
HbsAg	1 (3.3%)	0 (0%)	1 (1.7%)	2.472	>0.05
HCV Ab and HbsAg	3 (10%)	2 (6.7%)	5 (8.3%)	0.324	>0.05

There is no significant difference of the mean values between the studied groups.

Table (3): Comparison between the studied groups as regard AFP values before treatment (cut-off value 200 ng/ml)

AFP(ng/ml)	Group I	Group II	Total	T	P
Positive	23 (76.6%)	21 (70%)	44 (73.3%)		
Negative	7 (23.3%)	9 (3%)	16 (26.6%)		
Mean ± SD	781.46±667.27	575.00±406.86	662.16±563.16	1.683	>0.05
Range	118.0– 2965.0	103.0-1382.0	103.0 – 2965.0		

There is no significant difference of the mean values between the studied groups.

Table (4): Comparison between AFP values before treatment, 1 and 3 months after treatment

	Group I	Group II	Total	T	P
	Mean± S. D	Mean± S. D			
Before treatment	781.46± 667.27	575.00± 406.86	678.23±537.065	5.363	>0.05
1 month after	246.26± 164.46	342.83± 237.87	294.545±201.165	0.609	<0.05
3 months after	223.40± 128.42	318.90± 196.45	271.15±162.435	0.425	>0.05

There is a significant difference of the mean values between the studied groups after 1 months after treatment.

Table (5): Comparison between two procedures for HCC ablation in two groups of patients 5.1-7 cm in maximum diameter based on triphasic CT 1 month after ablation

Response	Group I	Group II	Total	x ²	p-value
	(n=30)	(n=30)			
Complete ablation	18(60%)	25(83.3%)	43(71.6%)	4.955	<0.05
Incomplete ablation	12(40%)	5(16.7%)	17(28.3%)		

There is a significant difference of the mean values between the studied groups.

Table (6): Comparison of two procedures for HCC ablation in two groups of patients 5.1-7 cm in maximum diameter based on triphasic CT 3 months after ablation

Response	Group I	Group II	Total	x ²	p-value
	(n=30)	(n=30)			
Complete ablation	17(56.7%)	24(80%)	41(68.3%)	4.274	<0.05
Incomplete ablation	13(43.3%)	6(20%)	19(31.6%)		

There is a significant difference of the mean values between the studied groups.

Table (7): Comparison of two procedures for HCC ablation in subgroups of patients 5.1-6 cm in maximum diameter based on triphasic CT after 3 months

Response	Group (I) Subgroup A	Group (II) Subgroup A	x ²	p-value
	(n=15)	(n=15)		
Complete ablation	10(66.7%)	13(86.6%)	4.065	<0.05
Incomplete ablation	5(33.3%)	2(13.3%)		

There is a significant difference of the mean values between the studied groups.

Table (8): Comparison of two procedures for HCC ablation in subgroups of patients 6.1-7 cm in maximum diameter based on triphasic CT after 3 months

Response	Group (I) Subgroup B	Group (II) Subgroup B	x ²	p-value
	(n=15)	(n=15)		
Complete ablation	7(46.7%)	12(80%)	4.218	<0.05
Incomplete ablation	8(53.3%)	3(20%)		

There is a significant difference of the mean values between the studied groups

Table (9): Comparison of the response between the two subgroups of G I after 1 month

Response	G I A (5.1-6)	G I B (6.1-7)	Total	χ^2	p-value
	(n=15)	(n=15)			
Complete ablation	10(66.7%)	8(53.3%)	18(60%)	0.540	>0.05
Incomplete ablation	5(33.3%)	7(46.6%)	12(40%)		

This table shows no statistically significant difference between the two subgroups.

Table (10): Comparison of the response between the two subgroups of G I after 3 months

Response	G I A (5.1-6)	G I B (6.1-7)	Total	χ^2	p-value
	(n=15)	(n=15)			
Complete ablation	10(66.7%)	7(46.6%)	17(56.6%)	0.564	>0.05
Incomplete ablation	5(33.3%)	8(53.3%)	13(43.3%)		

This table shows no statistically significant difference between the two subgroups.

Table (11): Comparison of the response between the two subgroups of G II after 1 month

Response	G II A (5.1-6) cm	G II B (6.1-7) cm	Total	χ^2	p-value
	(n=15)	(n=15)			
Complete ablation	13(86.6%)	12(80%)	25(83.3%)	0.099	>0.05
Incomplete ablation	2(13.3%)	3(20%)	5(16.7%)		

This table shows no statistically significant difference between the two subgroups. Complete ablation was detected in 83.3% of the cases after 1 month.

Table (12): Comparison of the response between the two subgroups of G II after 3 months

Response	G II A (5.1-6) cm	G II B (6.1-7) cm	Total	χ^2	p-value
	(n=15)	(n=15)			
Complete ablation	13(86.6%)	11(73.3%)	24(80%)	0.095	>0.05
Incomplete ablation	2(13.3%)	4(26.7%)	6(20%)		

This table shows no statistically significant difference between the two subgroups.

Table (13): Comparison between both groups in post-procedural complications

Response	GI	GII	Total	χ^2	p-value
	(n=30)	(n=30)			
Abdominal pain	18(60%)	20(66.7%)	38(63.3%)	1.087	>0.05
Fever	7(23.3%)	9(30%)	16(26.6%)		

This table shows no statistically significant difference between the two subgroups



Figure (1): Triphasic CT; arterial phase, shows early enhanced well-defined HCC measuring about 6.5x5 cm in diameter in the right hepatic lobe.

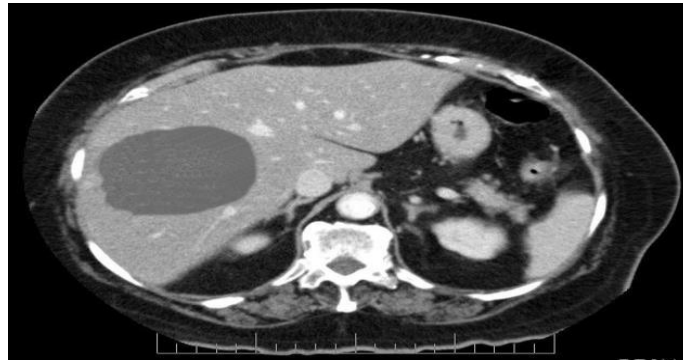


Figure (2): Follow up CT scan 1-month post RFA+PAI shows a well-defined hypodense lesion in the site of the ablated tumor (6.5x5 cm) which does not enhance in the arterial phase.

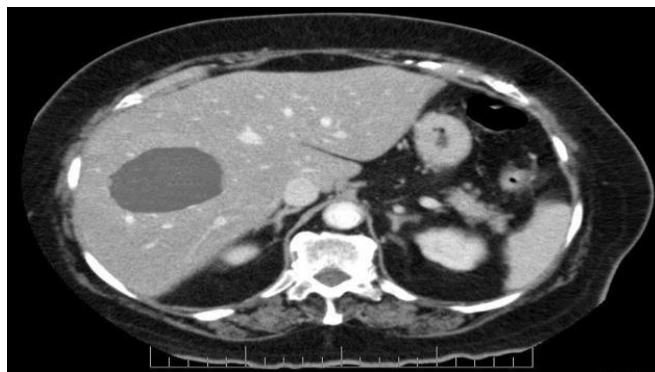


Figure (3): Follow up CT scan 3- months post RFA+PAI, decrease in the size of the lesion with complete ablation which does not enhance in the arterial phase.

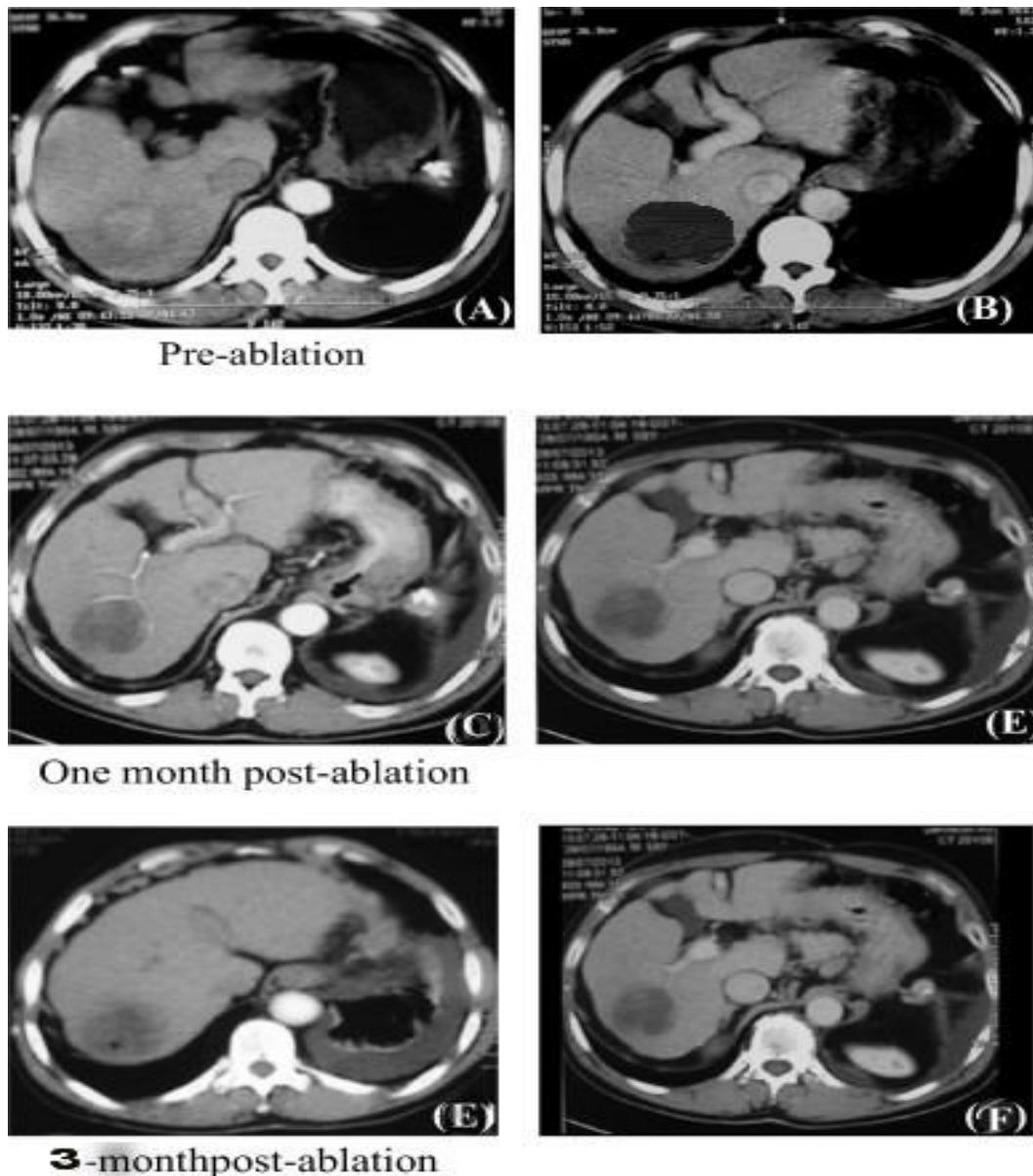


Figure (4): Triphasic CT; arterial phase on left and delayed phase on right, shows early enhanced well-defined HCC measuring about 6x5 cm in the right hepatic lobe. 1-month and 3-month post ablation using RFA+PAI shows complete ablation.

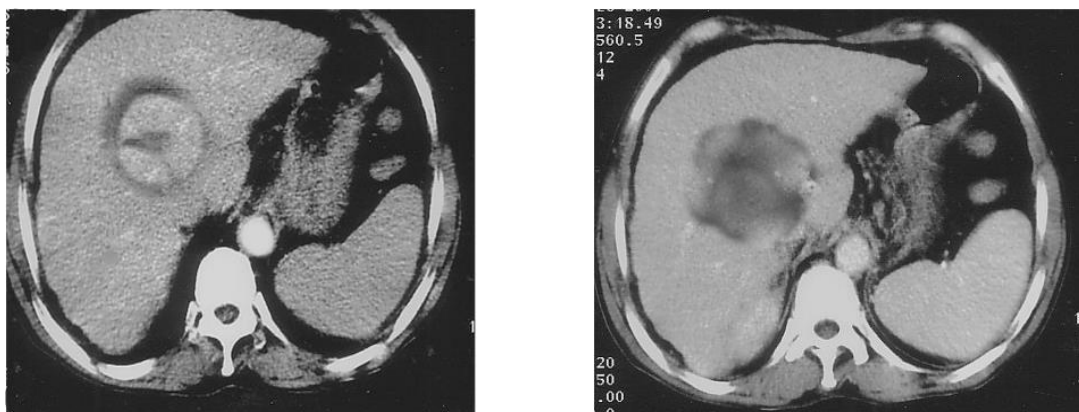


Figure (5): Triphasic CT scan (left) pre RFA+PEI shows a HCC (5.4x5.2cm). The 3-months CT scan (right) shows partial ablation of the lesion.

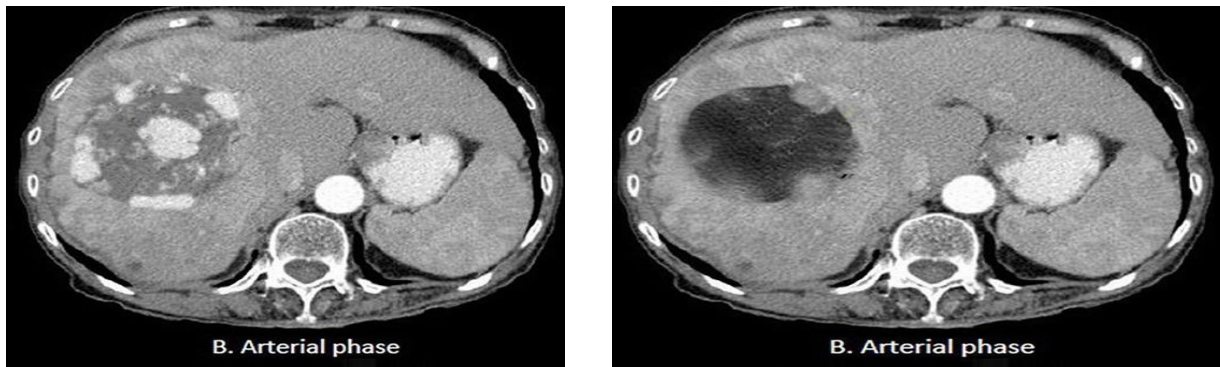


Figure (6): Triphasic CT scan (left) pre RFA+PEI shows a HCC (6x5.8cm). The 3-months CT scan (right) shows partial ablation of the lesion.

DISCUSSION

This study was conducted on 60 HCC patients to test the effectiveness of two modalities on large lesions, 5.1-7 cm in the largest diameter. All the patients were Child A and this was a part of the inclusion criteria to achieve high success rate with the least complication. Males were predominant with a ratio of 5.7:1. This ratio is similar to that reported by Sherlock and Dooley [10] who pronounced male predominance of HCC throughout the world, on the order of 4:1 in low incidence area and up to 9:1 in high incidence areas. This ratio could be explained by hormonal factors as high testosterone level in males and methoxy-estradiol in females [11]. The mean age was 60.3 ± 8.6 and this was slightly higher than some Egyptian studies conducted by Azab et al. [12], Salama et al. [13] and El-Kady et al. [14], probably because of the selection of patients, since the previous studies included patients with smaller lesions up to 5 cm compared to our study in which longer time was needed by the lesions to develop larger sizes. The mean age was slightly lower than the results of the Japanese and Italian patients conducted by Omata et al. [15] and Livraghi et al. [16] respectively. El-Zayadi et al. [17] reported that the average age of HCC patients was 60 years as was found in the study of Badawi and Michael [18]. The earlier age among Egyptians could be explained by the early age of acquisition of viral hepatitis (HCV and HBV) due to several factors can transmit infection among the population such as injections, circumcision and tattooing without proper sterilization [19].

In the present study AFP was positive in 73% of patients. In the follow up period the number of positive patients dropped to 20% and 15% one and three months later respectively. This was in agreement with Lawrence et al. [23] who stated

that not all HCCs secrete AFP and approximately 30% of the patients had normal AFP levels. In the current study the post-procedural complications were mild that included abdominal pain in 18(60%) and 20(66.7%) and fever in 7(23.3%) and 9 (30%) patients in **GI** and **GII** respectively. No significant statistical difference regarding complications in the two modalities in this study. Other complications including hematemesis, ascites were not observed. Livraghi et al. [16] reported hemothorax (2%) due to damage of an intercostal vessel along the needle track, intraperitoneal bleeding, hemobilia and mild cholecystitis. Poon et al. [24] reported complications as segmental bile duct injury and multi-organ failure. In another study by Livraghi et al. [25], two major complications including massive bleeding from the liver capsule and death. However, both complications appeared largely attributable to surgeon error rather than to the RFA procedure itself. Complete ablation was achieved in 43(71.6%) of all (60) patients; 18 (60%) in GI compared to 25 (83.3%) of GII, after 3 months as confirmed by triphasic CT with contrast. The difference was statically significant denoting better penetration of acetic acid as it destroys the fibrous septa present in the HCC. When the studied patients were stratified into subgroups A (5.1-6 cm) and B (6.1-7 cm), ablation was significantly better in GII A than GI A (86.6% vs 66.7%) and in G IIB than G IB (80% vs 46.7%) denoting that when acetic acid was added to RFA ablation was better than when ethyl alcohol was added indicating better diffusion to the unablated zones of the HCCs. There are few studies concerning combination therapies for large tumors, PAI alone was compared to RFA alone with medium and large-sized tumors, acetic acid showed comparable results (76% vs 73.7%) in a study conducted by El-Kady et al. [26]. Also the current

results are similar to Poon et al. [24] who conducted the first study in which the effectiveness of RFA of HCCs 3.1 to 8.0 cm in diameter by means of a predominantly surgical approach was evaluated specifically. He demonstrated an overall complete ablation rate of 91% for HCCs 3.1 to 8.0 cm in diameter and 93% for HCCs 3.1 to 5 cm. He assumed that radical ablation of large tumors is difficult to achieve percutaneously, even with repeated sessions. In the study conducted by El-Kady et al. [26], PAI alone was used for medium and large-sized tumors ablated 76% of the lesions. When PAI was compared to PEI in tumors up to 6 cm in diameter, ablation was achieved in 86.7% and 66.7% respectively [5]. Combined therapies in smaller tumors up to 5 cm gave better results than in large tumors. In one study conducted by Azab et al., [27] using RFA plus PEI, 97% of 33 lesions were ablated. In one study using RFA plus PAI ablation of large tumors (5-8 cm) conducted by Azab [28], ablation was achieved 46.6% compared to 20% in PAI alone. Another study using RFA plus PAI conducted by El-Sayed [29], ablation was achieved in 90% in lesion less than 5 cm. Our results showed the superiority RFA plus PAI combination for large tumors (>5 cm and up to 7 cm in diameter). Acetic acid has the ability to penetrate cells with the property of extracting collagen and dissolving lipids has a stronger killing effect on hepatocytes than ethanol as reported by Okada [30]. However more studies are needed to evaluate different combinations and frequency of sessions on larger series of patients.

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Conflicts of interest: None.

Ethical approval: Approved .

CONCLUSION

Ablation of inoperable large HCC 5.1-7cm can be done with combined techniques. RFA plus acetic acid was compared to RFA plus ethyl alcohol. Complete ablation was superior when acetic acid was used. This is probably due to the ability to penetrate cells with the property of extracting collagen and dissolving lipids leading to a stronger killing effect on hepatocytes. A study with larger number of patients and more frequent sessions of acetic acid injection after RFA may give better results.

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